Journal of Medical Virology 57:361–366 (1999)

Association of GB Virus C (GBV-C)/Hepatitis G Virus (HGV) With Haematological Diseases of Different Malignant Potential

Borislava G. Pavlova,[1,3]* Renate Heinz,[1,2] Ursula Selim,[2] Heinz Tüchler,[1] Elisabeth Pittermann,[2] and Gerald Eder[3]

[1]L. Boltzmann Institute for Leukemia Research and Haematology, Vienna, Austria
[2]3rd Medical Department, Hanusch Hospital, Vienna, Austria
[3]Hans Popper Primate Center, Baxter-Immuno, Orth/Donau, Austria

Among risk groups for GB virus C (GBV-C)/HGV infection, patients with haematological diseases are particularly exposed due to the combination of transfusional support and immunodeficiency status. To examine any association between GBV-C/HGV positivity and different malignancy potential of hematological diseases, we investigated two groups of patients, one with clonal stem cell disease with long latency period (myelodysplasia, myeloproliferative disease) and one with malignant haematological diseases (Hodgkin's lymphoma, non-Hodgkin's lymphoma, acute leukemia, multiple myeloma). Virus positivity was compared with the data from cytogenetic analysis at first diagnosis. The frequency of GBV-C/HGV infection in these patients was studied using reverse transcription-polymerase chain reaction (RT-PCR) and E2 antibody assay. Serum GBV-C RNA was found in 29/47 (62%) patients. The prevalence of GBV-C RNA in the group of oncological cases (72%) was significantly higher ($P = .02$) than in the patients with clonal stem cell diseases (28%). Among the GBV-C negative cases, only 25% had malignant haematological diseases. The data from GBV-C/HGV tested cases for which cytogenetic analysis was carried out indicated an association of GBV-C/HGV positivity with genomic destabilization in general. Of the cases with numerical and structural aberrations, 64% were GBV-C positive. A correlation could not be confirmed between GBV-C/HGV and liver enzyme levels, blood transfusions, chemotherapy treatment, or viral coinfection. These findings suggest a high risk of GBV-C/HGV infection in patients with haematological disorders especially in the group of malignant diseases. These observations may indicate that the persistence of GBV-C/HGV in these patients could be associated with susceptibility to genomic destabilisation. ***J. Med. Virol. 57: 361–366, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: GB virus C (GBV-C)/HGV; haematological malignancy; clonal stem cell diseases; cytogenetics

INTRODUCTION

GB virus-C (GBV-C), which has a genomic organization resembling that of the *Flaviviridae* virus family, was discovered by Simons et al. [1995] and another isolate (HGV) was identified subsequently by Linnen et al. [1996]. Because of the similarity of their nucleotides and amino acid sequences, it was concluded that GBV-C and HGV are variants of the same virus [Zuckerman, 1996]. Early reports indicated a high prevalence of GBV-C/HGV RNA in patients with chronic or acute hepatitis [Yoshiba et al., 1995; Heringlake et al., 1996; Sugai et al., 1997] and subjects with frequent parenteral exposure (blood transfusion recipients, drug addicts, hemophiliacs, and dialysis patients) [Dawson et al., 1996; Linnen et al., 1996; Schleicher et al., 1996; Tacke et al., 1997]. However, current evidence suggests that the spectrum of association of GBV-C/HGV infection with diseases involving organ systems other than the liver, ranges from aplastic anaemia [Byrnes et al., 1996; Zaidi et al., 1996; Moriyama et al., 1997]; human immunodeficiency virus (HIV)-positive idiopathic thrombocytopenia [Mattioli et al., 1996]; thalassemia [Zemel et al., 1998]; common variable immune deficiency [Webster et al., 1996]; and cryoglobulinemia [Tepper et al., 1998]. Among risk groups, patients with haematological diseases are particularly exposed due to the combination of frequent transfusional support and immunodeficient status. Thus, both the underlying

*Correspondence to: Borislava G. Pavlova, Ph.D., L. Boltzmann Institute for Leukemia Research and Haematology, Hanusch Hospital, H. Collinstrasse 30, A-1140 Vienna, Austria. E-mail: lbifluh@adis.at

Accepted 16 August 1998

disease and the therapy are thought to contribute to this prevalence. Reports of a higher frequency of GBV-C/HGV infection in multitransfused patients with haematological malignancy (48%) [Skidmore et al., 1997] compared with other groups examined [Alter HJ et al., 1997] suggest that GBV-C/HGV may be associated with the development of haematological diseases.

A closer relationship between the hepatitis C virus (HCV) and haematological lymphoproliferative disorders, especially mixed cryoglobulinaemia (MC), non-Hodgkin's lymphoma (NHL), multiple myeloma (MM), and monoclonal gammopathies of unknown significance (MGUS), shows that HCV is a possible factor or cofactor in the pathogenesis of these disorders [Silvestri and Baccarani, 1997]. Because the genome organization and the structural and biological characteristics of GBV-C/HGV are similar to that of HCV, it may also contribute to the development of haematological malignancy. To examine the hypothesis for association between GBV-C/HGV and different malignancy potential of haematological diseases, we investigated patients with clonal stem cell disease with long latency period (myelodysplasia [MDS], myeloproliferative disease [MPD]) and malignant haematological diseases (Hodgkin's lymphoma [MH], NHL, acute leukemia [AL], MM) and compared the data with that from cytogenetic analysis at first diagnosis. A previous history of blood transfusions, hepatitis virus coinfections, and immunosuppressive therapy as well as liver enzymes were analysed in the GBV-C-positive and -negative patient groups to investigate the association of GBV-C with various clinical characteristics.

MATERIALS AND METHODS

Patients

Serum samples were obtained from 47 haematological patients, 19 with clonal stem cell disease with long latency period and 28 with malignant hematological diseases. The study included 11 MDS, 5 MPD, 3 non-malignant haematological diseases, 3 MH, 13 NHL, 8 acute myeloid leukemias (AML), 2 acute lymphatic leukemias (ALL), and 2 MM. For each of the above patients, all available samples such as serum from other sampling dates and peripheral blood or bone marrow mononuclear cells (MNCs) were also tested. The cells were washed three times in phosphate-buffered saline (PBS) to avoid serum contamination and the supernatant from the last wash was conserved for further tests. The institutional ethics committee approved the trial and all the patients gave their written informed consent. The numbers and types of blood product transfusions (red blood cells, platelets, or plasma products); the liver enzyme levels including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γGT); the virus status; and the chemotherapy treatments that were recorded for each patient were also reviewed.

GBV-C and HCV Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Hybridization Analysis

To detect GBV-C RNA in a sample, nucleic acids were isolated from 100 μl of serum or 10^7 MNCs by the acid guanidinium thiocyanate-phenol-chloroform extraction method. The RT-PCR protocol used a nested PCR with degenerate primers for both the consensus sequence of the 5′ untranslated region (5′UTR) (external primer positions: 110–469, internal primer positions: 134–285) and the putative nonstructural region 3 (NS3) (external primer positions: 4326–4406, internal primer positions: 4342–4391) of the virus genome, to increase the sensitivity of detecting GBV-C and any of its variants. Each experiment was validated by use of known positive and negative control samples. Amplified products were analysed by electrophoresis through 3% NuSieve agarose gel (FMC, Rockland, MD). The sensitivity and specificity of the PCR results were further confirmed by dot blot hybridization with ^{32}P-labelled purified PCR amplicon from a known GBV-C-positive and previously sequenced sample used as probe [Kudo et al., 1997]. HCV RNA was detected by RT-PCR as described previously [Thaeler et al., 1991].

Serological testing for GBV-C, HCV, HBV, and HAV Markers

Serum samples from all patients were examined for antibodies to E2 envelope protein of GBV-C/HGV by an enzyme-linked immunosorbent assay (ELISA) developed recently (Boehringer Mannheim, Germany). Antibodies to HCV were sought by Ortho HCV 3.0 ELISA (Ortho Diagnostic Systems, Neckargmünd, Germany). The samples were also tested by IMX system assays available commercially (Abbott Laboratories, Chicago, IL) for hepatitis B virus surface antigen (HBsAg) and -antibodies (anti-HBs), and hepatitis A virus total antibodies (HAVAb).

Cytogenetic Analysis

Metaphases were prepared from MNCs according to standard techniques [Nowotny et al., 1996]; the tristaining technique was used for simultaneous production of reverse (R)- and DA-DAPI (C) - bands. The fluorescent dyes used were Chromomycin A3 (excitation wave length 435 nm) and DAPI (excitation wave length 360 nm) combined with Distamycin A as enhancer. Twenty mitoses were evaluated from each sample.

Statistical Methods

The Chi-square test was used for tests in contingency tables (in the case of 2 × 2 tables Yate's correction was applied). Group differences of continuous variables were tested by the Wilcoxon–Mann–Whitney U test. P values of less than .05 (two-sided) were considered to be statistically significant. For the prevalence of GBV-C/HGV a 95% confidence interval based on the binomial distribution was calculated.

TABLE I. Prevalence of GBV-C Infection Among Haematological Patients

Diagnosis	GBV-C-RNA positive (n = 29) (62%)	GBV-C-RNA negative (n = 18) (38%)	Total (n = 47)
Malignant haematological diseases	n = 21 (72%)	n = 7 (39%)	n = 28 (60%)
MH	2 (7%)	1 (6%)	3
NHL	10 (34%)	3 (17%)	13
AL	8 (28%)	2 (11%)	10
MM	1 (3%)	1 (6%)	2
Clonal stem cell disease with long latency period	n = 8 (28%)	n = 11 (61%)	n = 19 (40%)
MDS	3 (10%)	8 (44%)	11
MPD	4 (14%)	1 (6%)	5
Others	1[a] (3%)	2[b] (11%)	3

MH, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; AL, acute leukemia; MDS, myelodysplasia; MPD, myeloproliferative disease; MM, multiple myeloma.
[a]Thrombocytopenia.
[b]Aplastic anemia and leucopenia.

RESULTS

Prevalence of GBV-C in Haematological Patients With Malignant and Clonal Stem Cell Diseases

GBV-C RNA was found in serum samples of 29/47 (62%) (95% confidence interval, 45–75%) haematological patients. All 29 patients were RT-PCR positive by primers specific for the NS3 region, whilst not all cases showed positivity by primers for the 5′ noncoding region (NCR). RT-PCR results indicated that GBV-C RNA remained detectable with NS3 primers in all serial samples obtained from these patients within a period of 12 months. In addition, MNCs that were available from 13 serum GBV-C RNA-positive patients were also positive for viral RNA, even after the cells had been washed three times with PBS. The lack of viral RNA in the supernatant from the last cell wash suggested that the GBV-C RNA-positive signals were not due to serum contamination. The results were confirmed at least twice in repeated RT-PCR tests. The specificity of amplified fragments was confirmed further by hybridization of dot blotted RT-PCR products with ^{32}P-labelled viral sequence target sites.

The data of GBV-C-positive and -negative cases that are depicted in Table I indicate an association of the virus with malignant haematological diseases in general. The prevalence of GBV-C RNA in the group of neoplastic cases (72%) was significantly higher (P = .02) than in the patients with clonal stem cell diseases (28%). Among the neoplastic hematological diseases only 7/28 (25%) were GBV-C-negative cases. In addition, we found that decreased total lymphocyte counts (less than 800/μl) were significantly associated with the GBV-C positive cases (P = .034). The serological analysis (ELISA) showed that antibodies to E2 were detectable in 5/29 GBV-C RNA-positive and in only 1/18 GBV-C RNA-negative samples.

Of the 27 patients for which cytogenetic analyses were available, 12 had clonal stem cell disease with long latency period and 15 had malignant haematological diseases. The data from patients with GBV-C for which cytogenetic analysis was carried out indicated an association of GBV-C positivity with genomic destabilization in general (Table II). Of the 14/27 cases with a variety of numerical and structural aberrations, 9/14 (64%) were from GBV-C-positive and 5/14 (36%) were from GBV-C-negative patients. High numbers of nonclonal and single cell abnormalities were found in the group of GBV-C-positive cases. The chromosomal aberrations in the GBV-C-positive and -negative patients are heterogeneous and it is difficult to demonstrate any significant virus association.

Clinical Features of GBV-C-Positive and -Negative Cases

Of the 47 patients, 32 (68%) had received blood-component transfusions (erythrocyte and platelet concentrates) and blood products as supportive treatment of the haematological malignant disease; 22/32 (69%) were HGV RNA positive. Presence of HGV RNA was also found in 7/15 (47%) patients without a documented history of blood transfusions, but the difference between the virus-positive and -negative groups with respect to blood component transfusions was not significant (P = .184) (Table III). At the time of sampling, 22/47 (47%) patients were exposed continuously to immunosuppressive drug therapy. Sixteen of 22 (73%) immunosuppressed patients and 13/25 (52%) nontreated patients were GBV-C positive. These differences in the proportions may be seen as a trend towards high prevalence of GBV-C RNA in the chemotherapy-treated patients compared with that in the nonimmunosuppressed patients.

The patients infected with GBV-C had serum liver enzyme levels similar to those of patients without GBV-C infection (Table IV). There was no significant difference between the GBV-C-positive and -negative groups with respect to AST, ALT, and γGT values. Except for the one patient coinfected with HCV, all the GBV-C-positive patients showed no biochemical or clinical evidence of liver disease.

Among the 29 GBV-C RNA-positive patients, HCV RNA and HCV-Ab were detected in 1. Table V outlines the GBV-C genome-positive patients coinfected with other hepatitis viruses (HCV, HBV, HAV). On analysis of viral coinfection, no significant differences between

TABLE II. Cytogenetics of GBV-C Positive and -Negative Cases

Diagnosis (cytogenetic analysis available)	GBV-C positivity (%)	Cytogenetic abnormalities (%)
Malignant haematological diseases (n = 15)[a]	12/15[a]	8/15[a]
NHL (4[a]/13)	3/4[a] (75%)	4/4[a] (100%)
AL (9[a]/10)	8/9[a] (89%)	4/9[a] (44%)
MM (2[a]/2)	1/2[a] (50%)	0/2[a] (0%)
Clonal stem cell disease with long latency period (n = 12)[a]	5/12[a]	6/12[a]
MDS (8[a]/11)	3/8[a] (38%)	5/8[a] (63%)
MPD (3[a]/5)	2/3[a] (67%)	1/3[a] (33%)
Others[b] (1[a]/3)	0/1[a] (0%)	0/1[a] (0%)
Total[a]	17/27[a]	14/27[a]

MH, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; AL, acute leukemia; MDS, myelodysplasia; MPD, myeloproliferative disease; MM, multiple myeloma.
[a]Cytogenetic analysis available.
[b]Thrombocytopenia, aplastic anemia, and leucopenia.

TABLE III. Factors Associated With a Risk of GBV-C Infection

Factor	GBV-C RNA positive (n = 29) (62%)	GBV-C RNA negative (n = 18) (39%)	Total (n = 47)	P
No history of blood and plasma product use	7 (24%)	8 (44%)	15	.314
History of blood and plasma product use	22 (76%)	10 (56%)	32	.184
Erythrocyte units	22 (76%)	10 (56%)	32	.184
Platelet units	9 (31%)	2 (11%)	11	.201
Plasma products	5 (17%)	5 (28%)	10	.667
Chemotherapy	16 (57%)	6(33%)	22	.202

TABLE IV. Serum Liver Enzyme Levels of Haematological Patients According to Their GBV-C RNA Status

Liver enzymes	GBV-C RNA positive (n = 29) (62%)	GBV-C RNA negative (n = 18) (39%)	Total (n = 47)	P
AST >18 U/II	7 (24%)	5 (28%)	12	1.0
ALT >22 U/I	9 (31%)	5 (28%)	14	1.0
γ GT >28 U/I	14 (48%)	7 (39%)	21	.842
ΣAST ± ALT ± γGT elevated	15 (52%)	10 (56%)	25	1.0

AST, aspartate aminotransferase; ALT, alanine transaminase; γGT, gamma-glutamyl transpeptidase.

TABLE V. Virus Co-Infection According to the GBV-C RNA Status

Characteristic	GBV-C RNA positive (n = 29) (62%)	GBV-C RNA negative (n = 18) (39%)	Total (n = 47)	P
Virus co-infection				
HAV-Ab	19 (66%)	12 (67%)	31	1.0
HBs-Ag	0	0	0	—
Hbs-Ab	5 (17%)	6 (33%)	11	.397
HCV-Ab	1 (3%)	0	1	1.0
No virus co-infection	4 (14%)	0	4	.512

HAV-Ab, hepatitis A virus antibodies; HBs-Ag, hepatitis B virus surface-antigen; HBs-Ab, hepatitis B virus surface-antibodies; HCV-Ab, hepatitis C virus antibodies.

the GBV-C-positive and -negative patient group regarding the number of HAV-Ab- and HBs-Ab-positive cases was observed.

DISCUSSION

The data from the present study confirmed the high frequency of GBV-C infection in haematological patients and suggest that 62% of persons comprising the most common haematological disease groups, such as MDS, MPD, HD, NHL, AL, and MM, may be infected with GBV-C. Twenty-nine patients were positive for GBV-C as determined by RT-PCR using NS3 primers, but not all 29 showed positive signals with 5′ UTR primers. Thus, among our patients, the NS3 sequence was highly conserved. Consequently, primers from this region appeared to be more efficient in the identification of different GBV-C/HGV genotypes than those derived from the 5′ UTR region (exhibiting high sequence

divergence between different isolates) [Hsieh et al., 1997]. No clear pattern emerged, and GBV-C/HGV infection was found in heterogeneous groups of hematological diseases with possibly different aetiologies. However, it is also evident that some groups are much more likely to be associated with the infection. The prevalence of GBV-C RNA in the group of neoplastic cases (72%) was significantly higher ($P = .02$) than in the patients with clonal stem cell diseases (28%). Among the GBV-C negative cases, only 25% had malignant haematological diseases.

Based on a series of observations that MC can evolve into NHL or can be complicated by NHL, it was hypothesized that HCV may be involved in the progression of disease with long latency period to neoplasias [Silvestri and Baccarani, 1997]. It has been shown recently that both HCV core protein and NS3 are able to induce cells to transform into a tumourigenic phenotype, which could lead to clonal expansion [Ray et al., 1996]. Like HCV, GBV-C/HGV is a lymphotropic member of the *Flaviviridae* family [Leary et al., 1996] and the association between GBV-C/HGV infection and increased risk of lymphoproliferative and malignant disorders is possible.

The presence of GBV-C in MNCs from a variety of patients representing the spectrum of haematological disease groups described here suggests a possible involvement of the GBV-C/HGV in the proliferative process leading to malignant disease. Although the actual site of GBV-C/HGV replication remains to be discovered [Mellor et al., 1998], the findings of GBV-C RNA in peripheral blood and bone marrow cells in vivo, combined with evidence for an unusually high prevalence of GBV-C infection in serum, provides further support for the hypothesis of an extrahepatic tropism of the virus and an influence of such infection on the pathogenesis of haematological diseases. The data from GBV-C/HGV-tested cases for which cytogenetic analysis was carried out indicated an association of GBV-C/HGV positivity with genomic destabilization in general. Of the cases with a variety of numerical and structural aberrations, 64% were GBV-C positive. These observations may indicate that genetic factors, a mutational event, or the cooperation of GBV-C/HGV itself, could lead to the activation of certain oncogenes, resulting in neoplasia.

The humoral immune response to GBV-C envelope protein E2 is, in general, associated with prior GBV-C infection and loss of GBV-C RNA [Tacke et al., 1997]. However, simultaneous GBV-C RNA and anti-E2 positivity was found in immunosuppressed patients (HIV positive and liver transplanted) [Schleicher et al., 1997]. Our findings showed that 5 of 29 GBV-C RNA positive patients, but only 1 of the 18 cases with no detectable GBV-C RNA, were positive for anti-E2 antibodies. None of the 5 patients positive for both GBV-C RNA and E2 antibodies showed loss of GBV-C viraemia when followed up for 1 year. Thus, an immune response to E2 in these patients does not seem to have virus-neutralising activity. These findings are in accord with the interpretation of Schleicher et al. [1996] and suggest that haematological patients who develop a humoral anti-GBV-C response do not proceed to clear the virus, possibly due to their immunodeficiency status related to or caused by the underlying disease and chemotherapy.

Among the patients tested for GBV-C infection, 69% of transfused patients and 47% of patients who had received no transfusions or blood products were positive. Because the prevalence of GBV-C viraemia among blood donors is 1–3% [Linnen et al., 1996; Alter HJ et al., 1997], our results suggest that blood and blood product transfusions alone do not explain all cases of GBV-C infection in haematological patients.

The finding that none of our patients with GBV-C infection (except for one coinfected with HCV) had biochemical or clinical evidence of acute or chronic hepatitis is in keeping with previously published results, which noted that GBV-C causes no hepatic dysfunction [Alter MJ et al., 1997; Lin, 1997]. It has been suggested that patients coinfected with some hepatitis viruses are at risk for acquiring GBV-C [Miyakawa and Mayumi, 1997]. The data show that the prevalence of GBV-C RNA is not increased when found in association with HCV, HBV, and HAV coinfection.

Results of other studies suggest that it is likely that chemotherapy causes activation of a low number of what may initially be latent viruses, primarily as a result of immunosuppression [Kew and Kassianides, 1996; Kudo et al., 1996]. In our study, a correlation between GBV-C positivity and chemotherapy, which has also been proposed as a potential risk factor, could not be confirmed. Although 73% of the patients who had received chemotherapy were GBV-C positive, this percentage was not statistically significant.

The importance of some viruses as a probable cause of certain malignancies, particularly HCV in malignant lymphoproliferative disorders [Luppi et al., 1996], Epstein-Barr virus in Hodgkin's disease [Niedobitek, 1996], and HTLV- I in adult T-cell leukaemia [Manns and Blattner, 1991], has been well documented. It should also be noted that GBV-C/HGV infection was detected at a high rate in patients with hepatocellular carcinoma, but is also fairly common in extrahepatic malignancies [Muller et al., 1997; Toniutto et al., 1998]. Whether the decreased total lymphocyte counts in the GBV-C-positive group may be an indicator of possible viral replication in the lymphoid cells, remains hypothetical. Precisely which cell populations contain the virus and whether the virus permissivity is specific only to patients with a positive immunodeficiency status remains to be determined. Recent considerations suggest that GBV-C infection may also play a role as an exogenous stimulus to cell deregulative processes, and in combination with other factors (i.e., genetic, and/or environmental) may contribute to a multistep cell transformation involved in haematological malignancy. Identifying the virus in tumour cells and tissues could have important implications regarding disease aetiology. Several viruses have already been proposed

as putative infectious cofactors for malignant haematological diseases. The above data suggest that GBV-C may also be a contributing cofactor.

REFERENCES

Alter HJ, Nakatsuji Y, Melpolder J, Wages J, Wesley R, Shih JW, Kim J. 1997. The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. N Engl J Med 336:747–754.

Alter MJ, Gallagher M, Morris TT, Moyer LA, Meeks EL, Krawczynski K, Kim JP, Margolis HS. 1997. Acute non-A-E hepatitis in the United States and the role of hepatitis G virus infection. N Engl J Med 336:741–746.

Byrnes J, Banks T, Piatack M, Jungsuh J, Kim P. 1996. Hepatitis G-associated aplastic anaemia. Lancet 348:472.

Dawson GJ, Schlauder GG, Pilot-Mathias TJ, Thiele D, Leary TP, Murphy P, Rosenblatt JE, Simons JN, Martison FEA, Gutierrez RA, Lentino JR, Pachucki C, Muerhoff AS, Widell A, Tegtmeier G, Desai S, Mushavar IK. 1996. Prevalence studies of GBV virus-C infection using reverse transcriptase-polymerase chain reaction. J Med Virol 50:97–103.

Heringlake S, Osterkamp S, Trautwein C, Tillmann HL, Boker K, Muerhoff S, Mushahwar IK, Hunsmann G, Manns MP. 1996. Association between fulminant hepatic failure and a strain of GBV virus C. Lancet 384:1626–1629.

Hsieh SY, Yang PY, Chen HC, Liaw YF. 1997. Cloning and characterization of the extreme 5′-terminal sequences of the RNA genomes of GB virus C/ hepatitis G virus. Proc Natl Acad Sci USA 94:3206–3210.

Kew M, Kassianides Ch. 1996. HGV: hepatitis G virus or harmless G virus? Lancet 348:10.

Kudo T, Morishima T, Shibata M. 1997. Hepatitis G infection (letter). N Engl J Med 337:276.

Kudo T, Morishima T, Tsuzuki K, Orito E, Mizokami M. 1996. Hepatitis G virus in immunosuppressed paediatric allograft recipients (letter). Lancet 348:751.

Leary T, Muerhoff S, Simons JN, Pilot-Matias T, Erker J, Chalmers M, Schlauder G, Dawson G, Desai S, Mushahwar I. 1996. Sequence and genomic organization of GBV-C: a novel member of the *Flaviviridae* associated with human non-A-E hepatitis. J Med Virol 48:60–67.

Lin HJ. 1997. Hepatitis G virus. J Int Fed Clin Chem 9:27–30.

Linnen J, Wages J, Zhang-Keck Z-Y, Fry KT, Krawczynski KZ, Alter H, Koonin G, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JW-K, Young L, Piatak M, Hoover C, Fernandez J, Chen S, Zou JC, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Foung SKH, Thomas H, Bradley DW, Margolis H, Kim JP. 1996. Molecular cloning and disease association of hepatitis G virus: a transfusion transmissible agent. Science 271: 505–508.

Luppi M, Ferrari MG, Bonaccorsi G, Longo G, Narni F, Barrozzi P, Marasca R, Mussini C, Torelli G. 1996. Hepatitis C virus infection in subsets of neoplastic lymphoproliferations not associated with cryoglobulinemia. Leukemia 10:351–335.

Manns A, Blattner WA. 1991. The epidemiology of the human T-cell lymphotropic virus type I and type II: etiologic role in human disease. Transfusion 31:67–75.

Mellor J, Haydon G, Blair C, Livingstone W, Simmonds P. 1998. Low level or absent in vivo replication of hepatitis C virus and hepatitis G virus/GB virus C in peripheral blood mononuclear cells. J Gen Virol 79:705–714.

Miyakawa Y, Mayumi M. 1997. Hepatitis G virus—a true hepatitis virus or an accidental tourist. N Engl J Med 336:795–796.

Moriyama K, Okamura T, Nakano S: Hepatitis GB virus C genome in the serum of aplastic anaemia patients receiving frequent blood transfusions. 1997. Br J Haematol 96:864–867.

Muller C, Pfeffel F, Peck-Radosavljevic M, Petermann D, Oesterreicher C, Pidlich J . 1997. Prevalence of hepatitis G virus in patients with hepatocellular carcinoma. J Viral Hepatol 4:411–414.

Niedobitek G. 1996. The role of Epstein-Barr virus in the pathogenesis of Hodgkin's disease. Ann Oncol 7(4):11–17.

Nowotny H, Karlic H, Grüner H, Vesely M, Nader A, Heinz R. 1996. Cytogenetic findings in 175 patients indicate that items of the Kiel classification should not be disregarded in the REAL classification of lymphoid neoplasms. Ann Hematol 72:291–301.

Ray RB, Lagging LM, Meyer K, Ray R. 1996. Hepatitis C virus core protein cooperates with ras and transforms primary rat embryo fibroblast to tumorigenic phenotype. J Virol 70:4438–4443.

Schleicher S, Chaves RL, Dehmer T, Gregor M, Hess G, Flehmig B. 1996. Identification of GBV-C hepatitis G RNA in chronic hepatitis C patients. J Med Virol 50:71–74.

Schleicher S, Normann A, Gregor M, Hess G, Flehmig B. 1997. Hepatitis G infection (letter). Lancet 349:954–955.

Silvestri F, Baccarani M. 1997. Hepatitis C virus-related lymphomas. Br J Haematol 99:475–480.

Simons JN, Leary TP, Dawson GJ, Pilot-Matias T, Muerhoff SA, Schlauder GG, Desai SM, Mushahwar IK. 1995. Isolation of novel virus-like sequences associated with human hepatitis. Nat Med 1:564–569.

Skidmore SJ, Collingham KE, Harrison P, Neilson JR, Pillay D, Milligan DW. 1997. High prevalence of hepatitis G virus in bone marrow transplant recipients and patients treated for acute leukemia. Blood 89:3853–3856.

Sugai Y, Nakayama H, Fukuda M, Sawada N, Tanaka T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M. 1997. Infection with GB virus C in patients with chronic liver disease. J Med Virol 51:175–181.

Tacke M, Kiyosawa K, Stark K, Schlueter V, Ofenloch-Haehnle B, Hess G, Engel AM. 1997. Detection of antibodies to a putative hepatitis G envelope protein. Lancet 349:318–320.

Tepper JL, Feinmann SV, D'Costa L, Sooknannan R, Pruzanski W. 1998. Hepatitis G and hepatitis C RNA viruses coexisting in cryoglobulinemia. J Rheumatol 25:925–928.

Thaeler MM, Park CK, Landers DV, Wara DW, Houghton M, Veereman-Wauters G, Sweet RL, Han JH. 1991. Vertical transmission of hepatitis C virus. Lancet 338:17–18.

Toniutto P, Pirisi M, Fabris C, Bardus P, Soardo G, Vitulli D, Tisminetzky SG, Pacco P, Casparini V, Baralle F, Bartoli E. 1998. High prevalence of infection with hepatitis G virus in patients with hepatic and extrahepatic malignancies. J Hepatol 28:550–555.

Webster ADB, Morris A, Wang Y, Deacock S, Dusheiko GM, Harrison TJ. 1996. HGV/GBV-C in patients with primary immunodeficiency (abstract). Proceedings of IX Triennial International Symposium on Viral Hepatitis and Liver Disease. Rome: p 260.

Yoshiba M, Okamoto H, Mishiro S. 1995. Detection of the GBV-C hepatitis virus genome in serum from patients with fulminant hepatitis of unknown aetiology. Lancet 346:1131–1132.

Zaidi Y, Chapman CS, Myint S. 1996. Aplastic anaemia after HGV infection (letter). Lancet 348:471–472.

Zemel R, Dickman R, Tamary H, Bukh J, Zaizov R, Tur-Kaspa R. 1998. Viremia, genetic heterogeneity, and immunity to hepatitis G/GB-C virus in multiply transfused patients with thalassemia. Transfusion 38:301–306.

Zuckerman AJ. 1996. Alphabet of hepatitis viruses. Lancet 347:558–559.